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EVALUATION OF THE ACUTE TOXICITY OF SELECTED GROUNDWATER CONTAMINANTS

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NOTICES

TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Bruce O. Stuart

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Director, Toxic Hazards Division
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Acute dermal and inhalation toxicology studies were conducted on several selected groundwater contaminants to provide additional data for hazard evaluations by Air Force personnel. Four of the compounds; Tetrachloroethylene, 1,1,1-Trichloroethane, Chlorobenzene, and 1,1-Dichloroethylene were tested for acute dermal toxicity; 2,3-Dimethylphenol and 1,1-Dichloroethane were tested for acute 4-hour inhalation toxicity. Rabbits dermally treated with an upper limit dose of 2 ml/kg did not demonstrate symptoms of toxicity. Saturated vapors of 2,3-Dimethylphenol did not result in mortality among exposed rats after 4 hours. However, 4-hour inhalation exposures to 1,1-Dichloroethane predicted an LC50 of approximately 13,000 ppm when using male rats.

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PREFACE

The research covered in this report began in October 1985 and was completed in February 1986. The work described in this report was begun by the University of California, Department of Community and Environmental Medicine, Toxic Hazards Research Unit, at Wright-Patterson Air Force Base, OH, under Air Force Contract Number F33615-80-C-0512. M.K. Pinkerton served as the Contract Technical Monitor for the Air Force Harry G. Armstrong Aerospace Medical Research Laboratory (AAMRL). The studies were completed and this report was drafted by Northrop Services, Inc. – Environmental Sciences (NSI-ES), 101 Woodman Drive, Dayton, OH; NSI-ES has operated the Toxic Hazards Research Unit since 16 January 1986 under Air Force Contract Number F33615-85-C-0532. Lt. Col. Harvey J. Clewell III is presently the AAMRL Contract Technical Monitor. Although NSI-ES has no reason to question these data, the company makes no warranty, expressed or implied, and assumes no legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, products, or processes disclosed.

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A-1

TABLE OF CONTENTS

LIST OF TABLES

SECTION 1

INTRODUCTION

The Toxic Hazards Research Unit was requested to evaluate the acute toxicity of several groundwater contaminants. These were chosen from a list of groundwater contaminants reviewed for the U.S. Air Force by the A.D. Little Company (1985). Hazard evaluations of these compounds by Air Force personnel could not be completed because sufficient acute toxicity data were not available. Acute dermal toxicity data were lacking for tetrachloroethylene, 1,1,1-trichloroethane, chlorobenzene, and 1,1-dichloroethylene. Acute inhalation toxicity data were lacking for 2,3-dimethylphenol and 1,1-dichloroethane.

SECTION 2

MATERIALS AND METHODS

ANIMALS

Male, albino, New Zealand White rabbits, weighing between 2 and 3 kg, were purchased from Clerco Research Farms in Cincinnati, OH. Male Fischer 344 rats, weighing between 200 and 250 g, were purchased from Charles River Laboratories in Wilmington, MA. The animals were maintained on a photoperiod of 12 h light (beginning at 6 a.m.) and 12 h darkness. Food and water were available *ad libitum*. Quality control studies conducted by Air Force personnel during the quarantine period showed the animals to be in good health.

TEST COMPOUNDS

Table 1 describes the compounds tested in this study.

Quality control analyses were performed on all six compounds. The five chlorinated compounds were analyzed using a gas chromatograph (GC), which was equipped with a thermal conductivity detector to allow a better comparison of peak area/mass for percent-purity calculations in mixtures possibly containing both halogenated and nonhalogenated hydrocarbons. The compound 2,3-dimethylphenol (DMP), a solid, was listed by the supplier as 97% pure. However, a headspace vapor sample revealed a number of volatile-compound impurities. The concentration of volatile compounds was reduced to below the level of sensitivity by metering air through a column containing DMP for 45 min. Because the inhalation test was to be conducted to specifically gauge the effects of DMP, this method was used to remove the impurities from the test sample prior to

TABLE 1. TEST COMPOUNDS

Compound	CAS No.	Density	Formula Weight	Route of Testing
Tetrachloroethylene[a] (TRCE)	127-18-4	1.614	165.83	Dermal
1,1,1-Trichloroethane[a]	71-55-6	1.100	133.4	Dermal
Chlorobenzene[a]	108-90-7	1.100	112.56	Dermal
1,1-Dichloroethylene[b]	75-34-4	1.213	96.94	Dermal
2,3-Dimethylphenol[b] (DMP)	526-75-0	-	122.17	Inhalation
1,1-Dichloroethane[b,c]	75-34-3	1.177	98.96	Inhalation

[a] Purchased from J.T. Baker Chemical Co.
[b] Purchased from Aldrich Chemical Co.
[c] Contained approximately 3% dioxane as a stabilizer

conducting the inhalation exposure. The purified DMP was then analyzed using a GC equipped with a flame ionization detector. Approximately 0.05 mg of the solid material was injected into the GC using a syringe with a side cavitation. Table 2 shows the purity reported by the suppliers, as well as the purity determined chromatographically.

TABLE 2. COMPARISON BETWEEN THE REPORTED PURITY AND MEASURED PURITY OF COMPOUNDS USED IN GROUNDWATER STUDY

Compound	Purity (%)	
	Reported	Measured
Tetrachloroethylene (TRCE)	100.0	100.0
1,1,1-Trichloroethane	99.9	96.4
Chlorobenzene	99.9	99.9
1,1-Dichloroethylene	99.0	100.0
2,3-Dimethylphenol (DMP)	97.0	98.1
1,1-Dichloroethane	[a]	92.6

[a] No purity analysis provided by vendor.

DERMAL TOXICITY TESTING

Testing was initiated by dosing 5 rabbits with 2 mL test material/kg body weight. If there was no mortality at this level no additional dose levels would be tested. If mortality had been produced, testing would have continued with five animals per dose level.

Each rabbit's fur was shaved as closely as possible with an Oster® clipper equipped with surgical blades and a vacuum attachment. The rabbits' back and sides (about halfway down to the abdomen) were clipped from the saddle area of the shoulders to the top of the rear leg area. The

animals were individually weighed prior to dosing to determine the proper dose volume. Undiluted liquid material was applied to the back of the rabbit and in equal portions to the two sides. The compound was kept in place with 8-ply gauze patches. Clear plastic wrap was then applied to the entire clipped back area, and the entire midsection of the rabbit was wrapped with Elastoplast® tape. After a 24-h exposure to the compound, the tape, plastic wrap, and gauze were removed. The rabbits were housed in individual cages and observed for mortality or other signs of toxicity frequently on the day of dosing and twice daily thereafter. Clinical signs were recorded on symptomatology forms. Body weights were obtained at the time of dosing and on days 1, 2, 4, 7, 10, and 14 posttreatment.

In addition to the standard dermal test, blood levels of tetrachloroethylene (TRCE) were measured during and after the 24-h dermal contact. Five rabbits were exposed to the upper dosing limit of 2 mL/kg. Blood samples were taken from the rabbits' ears on an alternating schedule such that the same ear was never used for consecutive samplings. Samples were taken at 2, 4, 8, and 24 h during contact and at 4, 8, and 24 h and 2 and 7 days following exposure. To lessen anxiety during blood collection, rabbits were lightly sedated with an intramuscular injection of Innovar-Vet® (0.15 mL/kg).

INHALATION TESTING

Five male Fischer-344 rats weighing between 200 and 250 g were exposed to saturated vapors of DMP for 4 h in a clear, 120-L Plexiglas chamber. The concentration of DMP in the chamber was controlled by metering air through a column packed with pulverized DMP, and the chamber atmosphere was monitored until maximum concentration was achieved. The rats were then placed into the exposure chamber via a cage-drawer system that allows insertion of the cage with minimal loss of contaminant. Following the 4-h exposure, the rats were observed for 14 days. The rats were weighed at 1, 2, 4, 7, and 14 days postexposure, and the body weights were compared to those of control animals.

Four groups of five male Fischer-344 rats weighing between 195 and 262 g were exposed to several different concentrations of 1,1-dichloroethane for 4 h. This liquid agent was vaporized by metering it into an evaporator. A controlled airflow, which passed through the evaporator at a minimum of 28 L/min (20 chamber volumes/h), carried the vapor to a 60-L exposure chamber. The rats were observed during exposure and twice daily for 14 days. Body weights were obtained prior to exposure and at 1, 2, 3, 7, and 14 days postexposure. The LC_{50} of 1,1-dichloroethane was calculated from the number of deaths that occurred during the 14-day postexposure period using the moving average method of Weil (1952). Gross examinations of lung, liver, spleen, and brain were performed on each rat sacrificed at 14 days posttreatment.

ANALYSIS OF EXPOSURE ATMOSPHERES

A Miran 1A infrared analyzer (Foxboro) was used to monitor the concentration stability of DMP in the chamber, while the actual concentration of DMP was determined by GC analysis of impinger samples collected in water. The GC detector peak areas were compared to DMP standards in water.

The 1,1-dichloroethane concentration of the chamber was monitored continuously with a Miran 1A infrared analyzer equipped with an 8-cm gas cell. The absorbance data were converted to part-per-million values using linear regression calibration plots that were established using known atmospheric concentrations prepared in 5-L Mylar® bags. The exposure concentrations for individual studies were determined using standard absorbance data that closely bracketed the upper and lower ends of the scale of those observed during the exposure.

BLOOD ANALYSIS

Blood samples were taken from dermally exposed rabbits for assessment of TRCE levels. The presence of TRCE in the rabbits' blood was determined following extraction of TRCE from the blood with hexane. This extract was analyzed using a GC equipped with an electron capture detector. Therefore, the reported blood concentration results reflect extractable rather than total TRCE.

A standard curve was generated by comparing the area under the gas chromatogram curves to the concentration of TRCE in the extract. Test-data peak areas were converted to blood concentrations using a linear least-squares analysis over limited ranges of the standard curve that corresponded to, and bracketed, the test data. These conversions were also investigated by polynomial least-squares analysis, employing all of the standard data. No significant differences were observed in the blood concentration data generated using these two methods. All of the data presented were generated using the linear method

SECTION 3

RESULTS

DERMAL EXPOSURE

Dermal toxicity tests were conducted at a maximum upper dose limit of 2 mL/kg. None of the four compounds caused mortality. After an initial reduction in body weight, all rabbits demonstrated weight gains. A summary of the dermal toxicity data is provided in Table 3.

TABLE 3. RESULTS OF ACUTE DERMAL TOXICITY TESTING OF SELECTED GROUNDWATER CONTAMINANTS ON MALE RABBITS DOSED WITH 2mL/kg

Compound	14-Day Mortality Ratio
Tetrachloroethylene (TRCE)	0/5
1,1,1-Trichloroethane	0/5
Chlorobenzene	0/5
1,1-Dichloroethylene	0/5

Five rabbits were dermally exposed to 2 mL/kg of TRCE for 24 h. Serial blood samples were taken for TRCE determinations at various time periods throughout 7 days following the exposure. None of the rabbits died as a result of the TRCE exposure. One rabbit sustained an injury and had to be euthanized after the 4-h posttreatment blood sample was taken. The results of the blood analyses are provided in Table 4. Preexposure or control rabbit blood values were not determined during these studies. However, a sample of blood drawn from an untreated rabbit before this study began did not reveal a peak at the retention time characteristic of TRCE.

TABLE 4. TETRACHLOROETHYLENE CONCENTRATIONS IN RABBIT BLOOD DURING AND AFTER DERMAL CONTACT OF 2 mL/kg

	Sampling Time	TRCE µg/mL Mean (N = 5) ± S.D.
During Exposure	2 h	2.361 ± 0.844
	4 h	1.107 ± 0.323
	8 h	0.693 ± 0.285
	24 h	0.243 ± 0.139
Postexposure	4 h	0.304 ± 0.088
	8 h	0.354[a] ± 0.147
	24 h	0.110[a] ± 0.020
	2 days	0.083[a] ± 0.045
	7 days	0.015[a] ± 0.007

[a] N = 4

INHALATION EXPOSURE

Five male Fischer-344 rats were exposed by inhalation to a mean concentration of 85.5 mg/m^3 DMP for 4 h. None of the rats died during the exposure or during the 14-day observation period. Mean gains in body weight of the exposed rats during the postexposure period compared favorably with the weight gains of the control rats. Gross pathologic examination of the rats following the 14-day postexposure period revealed no exposure-related lesions.

Four groups of five rats each were exposed to various concentrations of 1,1-dichloroethane for 4-h periods. The concentrations and mortality data are provided in Table 5. All rats were rapidly

anesthetized by 1,1-dichloroethane exposure at each concentration tested. All of the recorded deaths occurred during the exposure period. All rats that survived the 4-h exposure period also survived through the 14-day observation period. Gross pathological examination of the rats that died following exposure revealed congested lungs and livers. Examination of the rats that survived revealed no exposure-related lesions.

TABLE 5. MORTALITY OF MALE RATS AFTER FOUR-HOUR INHALATION EXPOSURES TO 1,1-DICHLOROETHANE

Concentration (ppm)	Mortality Ratio (N = 5)
23,886	5/5
18,353[a]	5/5
17,214	0/5
9,298[a]	0/5

[a] Concentrations rounded to 9,200 and 18,400 for calculation of LC_{50} by the Weil method.

An LC_{50} of approximately 13,000 ppm was calculated from the mortality data using the moving average method of Weil (1952). Confidence limits were not calculated because all exposures resulted in either zero or 100% mortality.

SECTION 4

DISCUSSION

The four compounds tested for dermal toxicity (at an upper limit of 2 mL/kg) caused no mortality following 24 h of contact and a 14-day observation period. These compounds are considered to be of a low order of toxicity by this route of exposure.

TRCE was rapidly absorbed and excreted (largely by the lungs; Pegg *et al.*, 1979) within the first 8 h of the 24-h contact period. Very little of the TRCE remained in the blood after 24 h.

DMP was shown to be nontoxic after a 4-h inhalation exposure to essentially saturated vapors. No signs of toxic stress were noted in the rats either during or following exposure.

Four-hour inhalation exposures of rats to 1,1-dichloroethane resulted in an LC_{50} of approximately 13,000 ppm. These data are in agreement with a report by Smyth (1956), which stated that rats survived 8 h after a 400-ppm inhalation exposure to 1,1-dichloroethane, but died after receiving a 16,000-ppm dose.

SECTION 5

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APPENDIX

QUALITY ASSURANCE VERIFICATION

Quality Assurance has determined by review process that the report entitled:

Evaluation of the Acute Toxicity of Selected Groundwater Contaminants

accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of this study.

Quality Assurance inspections of this study occurred on the dates given below:

November 21, 1985
May 6, 1986
October 7, 1986

Mathias G. Schneider Jr

Mathias G. Schneider
Quality Assurance Coordinator
Toxic Hazards Research Unit
Date: October 20, 1986